DECLARATION OF DR. PIERRETTE GAUDREAU

1. My name is Dr. Pierrette Gaudreau. I am a full professor in the Department of Medicine at the University of Montreal. One of my areas of expertise is in the characterization, function and regulation of the pituitary and renal growth hormone-releasing hormone receptor and its isoforms. I have worked as a professor and consultant in the field of growth hormone-releasing hormone for over 20 years.

2. In the field of research for GHRH analogues, it is well accepted that an *in vitro* rat pituitary cell assay is used as a screen to select the most active GHRH analogues for further testing. Numerous papers have been published that attest to this result.

3. A paper which shows the use of an *in vitro* rat pituitary cell assay as a standard test for the GHRH analogues is the paper to Lance et al. entitled "Super-active analogs of growth hormone-releasing factor (1-29)-amide" *Biochem Biophys Res Commun.* 1984;119(1):265-272 ("Lance"). Lance is directed to the study of various hGHRH analogues. The hGHRH analogues were tested in an *in vitro* rat pituitary cell test and in *in vivo* assays in rats and pigs. Lance notes that:

> [D-Ala^2]hpGRF(1-29)-NH_2 exhibits extremely high GH-releasing activity both in the pig (Fig. 1) and the rat (tables 1 and 3) and is also 50 times as potent as the parent peptide when tested in rat pituitary cell monolayer culture... Although, again, increased biological stability may be a factor, the in vitro data and time course experiments indicate that this high activity is probably the result of increased receptor affinity.
> (Lance, pg. 269)

Lance also states:

> The fact that the D-Ala^2 analog is almost as potent in the pig as in the rat suggests that this analog may be equally potent in humans.

Lance shows that an *in vitro* rat pituitary cell assay was considered a standard test for testing GHRH analogues as potential therapeutic agents. Lance also shows that positive

results from *in vitro* rat pituitary cell assays suggest that the GHRH analogues may be potent in humans.

4. Another paper which shows the use of an *in vitro* rat pituitary cell assay as a standard test for the GHRH analogues is the paper to Felix et al. entitled "Synthesis, biological activity and conformational analysis of cyclic GRF analogs" *Int J Pep Protein Res.* 1988;32(6):441-454 ("Felix"). In Felix, various cyclic hGHRH analogues, (referred to as "GRF analogs" in Felix), were studied. The various cyclic hGHRH analogues were tested using an *in vitro* rat pituitary cell assay (see Felix, pg. 450). The results of testing various analogues in the *in vitro* rat pituitary cell assay are shown in Table 2 (See Felix, pg. 444).

Felix shows that an *in vitro* rat pituitary cell assay was considered a standard test for testing GHRH analogues as potential therapeutic agents. Only the compounds showing a relative *in vitro* potency, in the rat pituitary cell assay, that is greater than the natural hGHRH (noted as GRF(1-29)NH_2 in Table 2 of Felix, see pg.) were selected for *in vivo* testing by Felix. None of the compounds having a relative *in vitro* potency, in the rat pituitary cell assay, that was less than or equal to that of natural hGHRH were used in the *in vivo* testing of Felix.

5. Another paper which shows the use of an *in vitro* rat pituitary cell assay as a standard test for the GHRH analogues is the paper to Zarandi et al. entitled "Synthesis and *in vitro* and *in vivo* activity of analogs of growth hormone-releasing hormone (GH-RH) with C-terminal agmatine" *Int J Pept Protein Res.* 1990;36(6):499-505 ("Zarandi 1990"). Zarandi 1990 is directed to the study of hGHRH analogues (referred to as "GHRH analogs" in Zarandi 1990). The various hGHRH analogues were tested using an *in vitro* rat pituitary cell assay (see Zarandi 1990, Tables 2 and 3, pgs 502, 503). Zarandi 1990 shows that an *in vitro* rat pituitary cell assay was considered a standard test for testing GHRH analogues as potential therapeutic agents. The compounds showing a relative

potency and binding affinity greater than that of natural hGHRH (e.g., $GHRH_{1\text{-}29}NH_2$) during *in vitro* testing using a rat pituitary cell assay were selected for *in vivo* testing by Zarandi 1990 (See Zarandi 1990, Table 4, pg. 504). Zarandi 1990 also shows that positive results from the *in vitro* tests are correlated with positive results for *in vivo* tests. None of the compounds having a relative *in vitro* potency, in the rat pituitary cell assay, that was less than or equal to that of natural hGHRH were used in the *in vivo* testing of Zarandi 1990.

6. Another paper which shows the use of an *in vitro* rat pituitary cell assay as a standard test for the GHRH analogues is the paper to Campbell et al. entitled "GRF analogs and fragments: correlation between receptor binding, activity and structure" *Peptides*. 1991, 12(3):569-574 ("Campbell"). Campbell is directed to the study of hGHRH analogues (referred to as hGRF analogs in Campbell). The various hGHRH analogues were tested using an *in vitro* rat pituitary cell assay (see Campbell, Table 1, pg. 570). Campbell also notes a correlation between the *in vitro* results and *in vivo* results. Specifically, Campbell states:

> When combined with [Ala^{15}]-replacement, the resulting trisubstituted analog, [$desNH_2Tyr^1$,D-Ala^2,Ala^{15}]hGRF(1-29)-NH_2 (6) is highly potent (Table 1: 4-5 fold that of hGRF) and resistant to plasma DPP-IV and trypsin-like degradation in vitro (10). The high in vivo activity of this analog reported recently in pigs(3) and dairy cows (13) may indeed be the combined result of high receptor affinity and increased resistance to enzymatic degradation. (Campbell, pg. 574, Col. 2)

Campbell shows that an *in vitro* rat pituitary cell assay was considered a standard test for testing GHRH analogues as potential therapeutic agents. The compound showing the highest relative potency and binding affinity compared to natural hGHRH (for example, $GHRH1\text{-}29NH_2$ or $GHRH1\text{-}44NH_2$) during *in vitro* testing using a rat pituitary cell assay was also used for *in vivo* testing. Campbell shows that positive results from the *in vitro* rat pituitary cell assays are correlated with positive results for *in vivo* tests. None of the

compounds having a relative *in vitro* potency, in the rat pituitary cell assay, that was less than or equal to that of natural hGHRH were reported as undergoing *in vivo* testing.

7. Another paper which shows the use of an *in vitro* rat pituitary cell assay as a standard test for the GHRH analogues is the paper to Zarandi et al. entitled "Potent agonists of growth hormone-releasing hormone. Part I" *Int J Pept Protein Res.* 1992;39(3):211-217. ("Zarandi 1992"). Zarandi 1992 is directed to the study of hGHRH analogues (referred to as GH-RH analogs in Zarandi 1992). The various hGHRH analogues were tested using an *in vitro* rat pituitary cell assay (see Zarandi 1992, Table 1, pg. 214). Zarandi 1992 shows that an *in vitro* rat pituitary cell assay was considered a standard test for testing GHRH analogues as potential therapeutic agents. The compounds showing significant *in vitro* relative potency using a rat pituitary cell assay (specifically, MZ-3-149, MZ-3-191, and MZ-3-201) were selected for *in vivo* testing by Zarandi 1992 (See Zarandi 1992, Tables 2 and 3, pgs. 215 and 216). Zarandi 1992 shows that positive results from the *in vitro* tests are correlated with positive results for *in vivo* tests. None of the compounds having a relative *in vitro* potency, in the rat pituitary cell assay, that was less than or equal to that of natural hGHRH (e.g., $GHRH_{1\text{-}29}NH_2$) were used in the *in vivo* testing of Zarandi 1992.

8. Another paper which shows the use of an *in vitro* rat pituitary cell assay as a standard test for the GHRH analogues is the paper to Izdebski et al. entitled "Synthesis and biological evaluation of superactive agonists of growth hormone-releasing hormone." *Proc Natl Acad Sci U.S.A.* 1995; 92(11):4872-4876 ("Izdebski 1995"). Izdebski 1995 is directed to the study of hGHRH analogues (referred to as hGH-RH analogues in Izdebski 1995). The various hGHRH analogues were tested using an *in vitro* rat pituitary cell assay (see Izdebski 1995, Table 2, pg. 4873). With respect to the *in vitro* results Izdebski 1995 notes that:

> In vitro GH-releasing activities of the peptides were studied in the superfused rat pituitary cell system. All peptides showed higher activity than hGH-RH-(1-29)-NH_2.
> (Izdebski 1995, Col. 1, pg. 4875)

Izdebski 1995 tested each of these analogues *in vivo* and noted that:

> The relative potencies of analogs JI-34, JI-36, JI-38, and JI-22 were 80.9, 95.8, 71.4, and 44.6 times higher, respectively, compared with hGH-RH-(1-29)-NH_2 (accepted as 1.0) at 15 min. and 89.7, 87.9, 116.8, and 217.7 times higher, respectively, at 30 min. after the injection. The potencies of the other analogs, although lower than those listed above, were still very high compared with the standard.
> (Izdebski 1995, Col. 2, pg. 4875, See also Table 3)

Izdebski 1995 shows that an *in vitro* rat pituitary cell assay was considered a standard test for testing GHRH analogues as potential therapeutic agents. Since all the compounds showed a higher relative potency compared to natural hGHRH (e.g., $GHRH_{1\text{-}29}NH_2$) during *in vitro* testing using a rat pituitary cell assay, all of the compounds were also used for *in vivo* testing. Izdebski 1995 shows that positive results from the *in vitro* tests are correlated with positive results for *in vivo* tests.

9. Another paper which shows the use of an *in vitro* rat pituitary cell assay as a standard test for the GHRH analogues is the paper to Cervini et al. entitled "Human growth hormone-releasing hormone hGHRH(1-29)-NH_2: systematic structure-activity relationship studies" *J Med Chem.* 1998;41(5):717-727 ("Cervini 1998"). Cervini 1998 is directed to study of hGHRH analogues. The various hGHRH analogues prepared were tested using an *in vitro* rat pituitary cell assay (see Cervini 1998, Tables 1-4, pgs. 719, 720, 722, and 723). Cervini 1998 shows that an *in vitro* rat pituitary cell assay was considered a standard test for testing hGHRH analogue as potential therapeutic agents. Cervini 1998 did not

perform any *in vivo* testing, basing their conclusions solely on the results of the *in vitro* tests.

10. Another paper which shows the use of an *in vitro* rat pituitary cell assay as a standard test for the GHRH analogues is the paper to Varga et al. entitled "Increased activity of antagonists of growth hormone-releasing hormone substituted at positions 8, 9, and 10" *Proceedings of the National Academy of Sciences of the United States of America.* 2004;101(6):1708-1713 ("Varga"). Varga is directed to the study of GHRH antagonists. The various GHRH antagonists were tested using an *in vitro* rat pituitary cell assay (see Varga, Tables 2 and 3, pg. 1710 and 1711). Varga shows that an *in vitro* rat pituitary cell assay was considered a standard test for testing GHRH analogues, in this case antagonists, as potential therapeutic agents. With respect to *in vivo* testing, Varga states:

> Some of the antagonists with high activity *in vitro* were also evaluated *in vivo* to assess their potency and duration of action. (Varga, pg. 1711, Col. 2, 2nd full paragraph)

Compounds showing a relative potency and binding affinity greater than that of a standard GHRH antagonist during *in vitro* testing using a rat pituitary cell assay were selected for *in vivo* testing by Varga (See Varga, Table 4, pg. 1712). Varga shows that positive results from the *in vitro* tests are used as the basis for selecting compounds to be used for *in vivo* tests.

11. Another paper which shows the use of an *in vitro* rat pituitary cell assay as a standard test for the GHRH analogues is the paper to Jette et al. entitled "Human Growth Hormone-Releasing Factor (hGRF)1-29-Albumin Bioconjugates Activate the GRF Receptor on the Anterior Pituitary in Rats: Identification of CJC-1295 as a Long-Lasting GRF Analog" *Endocrinology.* 2005;146(7):3052-3058 ("Jette"). Jette is directed to the study of hGHRH analogues (referred to as hGRF analogs in Jette). With respect to the study, Jette states:

> We initiated a research program to make a number of maleimido derivatives of $hGRF_{1-29}$ and hGRF analogs and thought that the rat physiology was deemed adequate to screen the products of *in vivo* bioconjugation with serum albumin. However, it has never been shown that a hGRF albumin conjugate or a fusion protein can activate the rat GRF receptor. For this reason, before initiating *in vivo* studies, the demonstration that a $hGRF_{1-29}$-albumin conjugate retains *in vitro* activity on cultured rat anterior pituitary cells becomes essential.
> (Jette, Col. 2, pg. 3052)

Jette shows that an *in vitro* rat pituitary cell assay was considered a standard test for testing GHRH analogues as potential therapeutic agents. Jette also shows that positive results from the *in vitro* tests were sufficient to justify further *in vivo* testing. For example, Jette states:

> The *in vitro* experiment on cultured rat anterior pituitary cells showed that all three HSA bioconjugates of modified or unmodified $hGRF_{1-29}$ amide are able to activate the rat GRF receptor in the nM range but not in the sub-pM range. The response in the presence of HSA was also measured, and no GH secretion was observed, indicating that the albumin alone could not activate the receptor. Although there was a loss of activity, relative to the free $hGRF_{1-29}$ amide, this experiment confirmed that the rat model could be used for our *in vivo* screen.
> (Jette, Col. 2, pg. 3056)

12. Another paper which shows the use of an *in vitro* rat pituitary cell assay as a standard test for the GHRH analogues is the paper to Zarandi et al. entitled "Lipopeptide antagonists of growth hormone-releasing hormone with improved antitumor activities" *Proceedings of the National Academy of Sciences of the United States of America.* 2006;103(12):4610-4615 ("Zarandi 2006"). Zarandi 2006 is directed to the study of hGHRH antagonists. The various GHRH antagonists were tested using an *in vitro* rat pituitary cell assay (see Zarandi 2006, Tables 2 and 3, pgs. 4611 and 4612). Zarandi 2006 shows that an *in vitro* rat pituitary cell assay was considered a standard test for testing GHRH analogues, in this

case antagonists, as potential therapeutic agents. Zarandi 2006 also performs *in vivo* tests. With respect to *in vivo* testing, Zarandi 2006 states:

> Some of the antagonists with high activity *in vitro* were also evaluated *in vivo* to assess their potency and duration of action.
> (Zarandi 2006, pg. 4612, Col. 1, 1st full paragraph)

Compounds showing a relative potency and binding affinity greater than that of a standard antagonist during *in vitro* testing using a rat pituitary cell assay were selected for *in vivo* testing by Zarandi 2006 (See Zarandi 2006, Table 4, pg. 4613). Zarandi 2006 also shows that positive results from the *in vitro* tests are correlated with positive results for *in vivo* tests. None of the compounds having a relative *in vitro* potency, in the rat pituitary cell assay, that was less than or equal to that of the standard antagonist were used in the *in vivo* testing of Zarandi 2006.

13. Another paper which shows the use of an *in vitro* rat pituitary cell assay as a standard test for the GHRH analogues is the paper to Izdebski et al. entitled "A method for evaluation of activity of growth hormone-releasing hormone analogues." *Neuro Endocrinol. Lett.* 2007;28(4):382-385 ("Izdebski 2007"). Izdebski 2007 is directed to a method for the preliminary evaluation of new hGHRH analogues (referred to as hGH-RH analogs in Izdebski 2007). Specifically, Izdebski 2007 describes a method in which the GHRH analogues are added to a pituitary rat cell culture, the medium collected at specified times, and the growth hormone concentration measured in each collected sample. Izdebski 2007 found that:

> It can be concluded that the described procedure may be useful for screening *in vitro* new compounds before more laborious and costly *in vivo* evaluation would be used.
> (Izdebski 2007, pg. 385)

Izdebski 2007 shows that an *in vitro* rat pituitary cell assay was considered a standard test for testing GHRH analogues as potential therapeutic agents.

14. The paper to Iruthayanathan et al. entitled "Dehydroepiandrosterone Restoration of Growth Hormone Gene Expression in Aging Female Rats, in Vivo and in Vitro: Evidence for Actions via Estrogen Receptors" *Endocrinology*. 2005;146(12):5176-5187 ("Iruthayanathan") describes the use of an *in vitro* rat pituitary cell assay for the study of the effects of hormones of growth hormone release. These tests were than followed up with *in vivo* testing in rats. Iruthayanathan shows that an *in vitro* rat pituitary cell assay was considered a standard test for testing the effects of hormones on GH release. Iruthayanathan also shows that positive results from the *in vitro* tests are used as the basis for selecting compounds to be used for *in vivo* tests.

15. I am the sole co-inventor of the subject matter described in U.S. Patent No. 5,854,216 (the "'216 Patent") entitled "Markers for Growth Hormone Releasing Factor Receptors." The subject matter described in the '216 Patent relates to markers for growth hormone-releasing hormone receptors that are superagonists and exhibit a greater resistance to proteolysis *in vitro* and *in vivo*.

16. During my research that lead to the filing of the '216 Patent, I synthesized analogues of human GHRH(1-29)NH_2 substituted with D- and/or Ala amino acids at specific positions. The substitutions were chosen to both maintain affinity of GHRH for the hGHRH receptor and produce peptide bonds that were less susceptible to proteolysis in serum and tissues (e.g., anterior pituitary, hypothalamus, liver, etc.)

17. Given the numerous publications, some of which are cited above, that attest to the correlation between rat pituitary receptor and human receptor activity, it was expected that the analogues that exhibited the highest binding affinity to the rat pituitary receptor would also exhibit equally high binding affinity to the human receptor. If this were otherwise, the numerous scientists from the listed papers would not have tested their GHRH analogues an *in vitro* rat pituitary cell assay prior to performing *in vivo* and human clinical trials.

18. Following the precedent of the numerous publications listed above, I tested the binding affinity of the synthesized analogues using an *in vitro* rat pituitary binding assay. The binding affinity for the rat pituitary receptor shows, in Table 11, that analogue 13 ([D-Ala^2, Ala^8, Ala^{15}, Lys^{22}] and analogue 7 [D-Ala^2, D-Tyr^{10}, Lys^{22}] have a stronger affinity for binding to rat pituitary GHRH receptor than the analogue 8 [D-Ala^2, D-Tyr^{10}, D-Ala^{15}, Lys^{22}]. Specifically the following relative binding affinities were found for some of the analogues, compared to human GHRH(1-29)NH_2 ("hGHRH(1-29)NH_2):

hGHRH(1-29)NH_2	100 ± 19
[D-Ala^2, Ala^8, Ala^{15}, Lys^{22}]	1333 ± 31
[D-Ala^2, D-Tyr^{10}, Lys^{22}]	490 ± 270
[D-Ala^2, D-Tyr^{10}, D-Ala^{15}, Lys^{22}]	104 ± 40

(See the '216 Patent, Table 11)

These results showed that certain analogues had a higher binding affinity in an *in vitro* rat pituitary cell assay compared to human GHRH(1-29)NH_2. Based on these results we performed further testing that showed that the *in vitro* results produced similar results during *in vivo* tests.

19. I am the sole inventor in U.S. Patent Application No. 10/527,598 (the '598 Application). It is my understanding that the application was rejected by the Examiner in view of the '216 Patent.

20. I tested various GHRH analogues prepared in conjunction with my earlier research on markers for growth hormone-releasing hormone receptors, for their binding affinity to the human GHRH receptor ("hGHRH-R") in stably transfected baby hamster kidney cells and the resistance to proteolysis in rat serum, human plasma and human serum. Following the precedent set by numerous other researchers, I mainly tested compounds that

exhibited a higher binding affinity compared to natural hGHRH during *in vitro* testing using a rat pituitary cell assay. I also tested a compound that exhibited a nearly equal binding affinity compared to natural hGHRH during *in vitro* testing using a rat pituitary binding assay.

21. The binding results of selected compounds to human GHRH receptor (hGHRH-R) in baby hamster kidney ("BHK") cells are shown in Table 8 of the '598 Application. Specifically the following relative binding affinities were found compared to human GHRH(1-29)NH_2:

hGHRH(1-29)NH_2	1
1. [D-Ala^2, Ala^8, Ala^{15}, Lys^{22}]	499±234
3. [D-Ala^2, D-Tyr^{10}, Lys^{22}]	239±55
5. [D-Ala^2, D-Tyr^{10}, D-Ala^{15}, Lys^{22}]	939±249

(See the '598 Patent Application, paragraph [0088], Table 8)

22. Analogues 1 and 3 follow the expected pattern seen during *in vitro* testing using a rat pituitary cell assay. Specifically, analogue 1 shows a higher binding to hGHRH-R than analogue 3, which is the same pattern seen in the rat model (See paragraph 18 above). Unexpectedly, analogue 5, which was the weakest binding of the three analogues listed during *in vitro* testing using a rat pituitary binding assay, exhibits the strongest binding affinity for the hGHRH-R model. Given that the first two analogues followed the binding affinity of the rat model, as would be expected based on the numerous publications, the result that analogue 5 exhibited a binding affinity to hGHRH-R that greatly surpassed the binding affinity of the other analogues was a surprising result.

23 In order to select compounds for use in humans, it is important that the compounds not only exhibited enhanced affinity to the GHRH-R, but also are resistant to degradation by the body, in order for the compounds to be able to produce their therapeutic effect. Thus,

even if a compound were to exhibit a strong binding affinity for hGHRH-R, if the compound does not exhibit a significant resistance to *in vivo* proteolysis, such a compound would be a poor candidate for use in the treatment of humans.

24. The relative resistance to *in vitro* proteolysis in human plasma of selected compounds is shown in Table 10 of the '598 Application. Specifically the relative resistance to proteolysis after 180-min incubation in human plasma, compared to human GHRH(1-29)NH_2, was found to be:

1. [D-Ala^2, Ala^8, Ala^{15}, Lys^{22}]	2.96±0.02
3. [D-Ala^2, D-Tyr^{10}, Lys^{22}]	3.54±0.23
5. [D-Ala^2, D-Tyr^{10}, D-Ala^{15}, Lys^{22}]	3.21±0.31
hGHRH(1-29)NH_2	1

(See the '598 Patent Application, paragraph [0090], Table 10)

25. The determination of an ideal candidate for use in human trials is based on a combination of the binding affinity to hGHRH-R and the resistance to proteolysis in human plasma. This combination is known as the "*in vitro* potency index." The *in vitro* potency index is determined by from multiplying i--the relative binding affinity of GHRH analogues compared with the native hGHRH (1-29)NH_2, in BHK cells expressing the hGHRH receptor; with ii--the relative resistance to *in vitro* proteolysis of compounds in comparison with hGHRH (1-29)NH_2 after preferably 60 or 180 minute-incubations in human plasma or human serum. (See the '598 Patent Application, paragraph [0040])

26. The *in vitro* potency index of the selected compounds is shown in Table 10 of the '598 Application. Specifically the *in vitro* potency index of the compounds was found to be:

1. [D-Ala^2, Ala^8, Ala^{15}, Lys^{22}]	1477
3. [D-Ala^2, D-Tyr^{10}, Lys^{22}]	846
5. [D-Ala^2, D-Tyr^{10}, D-Ala^{15}, Lys^{22}]	3014
hGHRH(1-29)NH_2	1

(See the '598 Patent Application, paragraph [0090], Table 10)

27. The simultaneous assaying of GHRH analogues for *in vitro* for binding to human GHRH receptor and for their stability in human plasma resulted in the unexpected selection of analogue 5 [D-Ala^2, D-Tyr^{10}, D-Ala^{15}, Lys^{22}] as our lead candidate for formulation in pharmaceutical compositions and use in human clinical trials. This result could not have been predicted from the results of the '216 Patent. As the inventor for both the '216 Patent and the '598 Application, this result was unexpected and represents a new discovery that could not have been predicted.

28. All statements made in this Declaration of my own knowledge are true and all statements made in this Declaration on information and belief are believed to be true, and these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both under 18 U.S.C. §1001 and may jeopardize the validity of this application or any patent issuing thereon.

Pierrette Gaudreau PhD.

November 2, 2009

Date

Pierrette Gaudreau PhD